

### **REMARKS/ARGUMENTS**

Reconsideration of this application is requested. Claims 22-39 are pending in the application. Of these claims 28 and 32-34 are indicated to contain allowable subject matter. While applicants appreciate the examiner's indication of allowable claims, they submit that the claims remaining in this application also define patentable subject matter and the prior art cited and applied against them is not of sufficient merit or pertinence to deny patentability of these claims. Accordingly, reconsideration is requested.

Responsive to items 1-2 of the Official Action, the dependencies of claims 36 and 37 have been amended to depend from claims 23 and 24, respectively. As the examiner will recognize, these claims correspond to PCT claims 2 and 3 and this Amendment simply corrects their dependency.

The present invention relates to an amperometric sensor which is suitable for determining the concentration of hydrogen peroxide in a sample. The sensor comprises a ferricyanide compound which, in reduced form, functions as a mediator selective for hydrogen peroxide.

The invention also provides a cartridge for an amperometric sensor suitable for measuring hydrogen peroxide in a sample, comprising the defined ferricyanide compound, as well as a method for determining the concentration of hydrogen peroxide or an analyte using the sensor. Preferred ferricyanide compounds are set out in claims 25 to 34, and in the description at page 3 line 18 to page 6 line 24. It is preferred that the ferricyanide compounds have a low solubility in water and aqueous phases in order to provide stability.

The amperometric sensor of the present invention works in a reductive mode, as explained at pages 7 to 8 of the application as originally filed. Under the applied potential, the oxidized form of the mediator at the working electrode is converted to the reduced form producing a diffusion limited current which can be measured and correlated with the concentration of analyte in a sample. This mode of operation allows an

electrode potential of about -400 mV to be used which does not result in any oxidation of interferants, so that the result is an accurate measurement of the amount of hydrogen peroxide in the sample which may be correlated to the amount of the analyte.

Rejecting various claims the Examiner cites a number of references and applicants provide the following comments, using the Examiner's paragraph numbering.

4. Chen *et al* provides a redox electrode for monitoring oxidase-catalyzed reactions. According to Chen, the potential of an electrode is measured, where  $\text{H}_2\text{O}_2$  oxidizes ferrocyanide thus changing the potential of the electrode. This is not an amperometric sensor. The principle of measurement used in the present invention is quite different. Hydrogen peroxide is reduced, and in the measurement method set out in the present application, the current flow associated with the reduction is measured, and may be correlated against the concentration of analyte in a sample.

5. Ikeda *et al* provides a method for trapping ionic mediators in the enzyme layer of an amperometric biosensor. A soluble ferri/ferro-cyanide mediator is trapped as part of an electrode assembly. It is not clear from the cited abstract that this can act as a mediator in the reduced form as is required by the present invention. The Examiner will also note that the abstract refers to the highly soluble ionic mediators K ferrocyanide and K ferricyanide. In the opinion of one of the inventors of the present application, these mediators should not be able to act in the reduced form, especially at -400 mV which is the preferred potential for operation of the amperometric sensors of the present invention.

6. The teaching of Yao *et al* and Tatsuma *et al* is similar, each requiring a peroxidase electrode. In the present application, the mediator is in the form of ferricyanide and is converted to ferrocyanide at preferably about -400 mV. The ferrocyanide is oxidized by hydrogen peroxide (generated by the enzyme) and this re-reduction of the mediator at a potential of -400 mV is the measurement signal in the present invention. In the Yao/Tatsuma cases, the catalytic effect of peroxidase is required to allow measurement that is then made at -50 mV. This potential is significantly different from that preferably used in the present invention. Further,

peroxidase is an essential component of each of the methods disclosed in Yao *et al* and Tatsuma *et al*, and the Examiner will note that peroxidase is not required for use in the present invention.

7/8. The Examiner cites abstracts of two articles by Zhdanov *et al*. The articles relate to amperometric titration of potassium ferricyanide with hydrogen peroxide at a potential of +1.0 v or -0.1 v. In the present invention, ferrocyanide is reacted with hydrogen peroxide generated by an enzyme reaction. This is a different reaction occurring at different potentials.

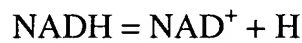
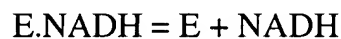
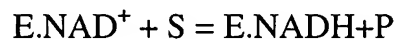
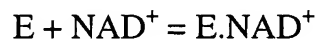
9. Blaedel *et al* provides a continuous analysis system which is a differential amperometric procedure, based on the continuous measurement of the rate of glucose oxidase reaction in a flowing system. Such a method would require a flowing stream to allow the measurement to be made. The mediator will also be soluble, and the measurement will be made at more positive potentials than the potentials preferred according to the present invention. The apparatus described by Blaedel *et al* is not a sensor.

10. Riffer provides an electrode for the potentiometric determination of dextran in a solution. This measurement uses a soluble mediator, and is not an amperometric measurement.

11. The Examiner cites Svitel *et al*, as evidenced by Baeza *et al* and Valdez *et al*. The Svitel *et al* article is well known to the inventors, and a cross-reference to this article is included in the description at page 6 in respect of preparation of the ferricyanide compounds. However, in Svitel *et al*, Baeza *et al* and in Valdez *et al*, the mediator is used in the oxidative mode, rather than the reductive mode as in the present invention. In Svitel *et al*, Baeza *et al* and Valdez *et al*, the oxidation current is being measured at positive potentials. In particular, according to the abstract of Svitel *et al*, a potential of +300 mV is used. There is no indication in any of the three references that the compounds can be used in the reductive mode as in the present invention, for the reduction of hydrogen peroxide. There are no figures (e.g. cyclic voltammograms) or

other data for the oxidation/reduction of mediators in the presence of hydrogen peroxide or the enzyme/substrate couple thus there is no indication in any of the three cited references of the possibility of reduction of hydrogen peroxide.

12. The Examiner cites Shiiki *et al* as evidenced by information on alcohol dehydrogenase obtained from a website. Shiiki *et al* provides an ethanol sensor consisting of a reference electrode and an enzyme, and an electrode acceptor-modified electrode containing an electrode agent of a mixture of an alcohol dehydrogenase and a hydrophobic electron acceptor. A dehydrogenase sensor acts in a completely different way from one using an oxidase. The dehydrogenase would use the following generalized reaction in the ethanol sensor of Shiiki *et al*:



where E represents enzyme, S represents substrate and P represents product.

The enzyme interacts with  $NAD^+$  which is the oxidized form of the mediator to provide  $E.NAD^+$  in which the oxidized form of the mediator is complexed with the enzyme. This complex then acts on the substrate to provide  $E.NADH$ , which is a complex of the reduced form of the mediator with the enzyme and the product.

It is important to note here that NAD measurements are made using an oxidized form of the mediator.

The Examiner recognizes that Shiiki *et al* does not refer to hydrogen peroxide, or to use in a reduced form as required for the present invention. The measurement potential used according to Shiiki *et al* precludes reductive measurements. Since there is no suggestion in Shiiki *et al* of reductive measurements, the present claims are novel.

13. The Examiner cites Nanba *et al* as evidenced by the information on dehydrogenases from the same website. Nanba *et al* discloses an enzyme sensor comprising an enzyme-modified electrode and a counter electrode, wherein the enzyme-

modified electrode comprises an electrode substrate and a homogeneous composition comprising an electron-transfer mediator and an enzyme on the surface of the electrode substrate. The enzyme and the electron transfer mediator interact to provide the homogeneous composition. It is quite clear from Nanba *et al* at column 1 lines 26 to 41 that the enzyme sensor does not include an oxidase, and the specific examples of Nanba *et al* all relate to the use of dehydrogenases. As explained above, the sensors of Nanba *et al* use an oxidative mode measurement, unlike those of the present invention.

In paragraphs 12 and 13 of the Action, the Examiner cites enzyme sensors which are in the oxidative mode in combination with information on dehydrogenase enzymes. The Examiner appears to be using hindsight to develop his objections of lack of novelty against the claims of the present application. There is no indication in either Shiiki *et al* or Nanba *et al* that the enzyme sensors considered in those references can be used in the reductive mode. The present claims are novel over the disclosure of these documents.

17-20. Claims 35 to 37 are rejected as being unpatentable over various combinations of documents. However, these claims refer to the use of the sensor of claim 22. Since it has been established above that claim 22 is novel, it follows that there is no motivation in any of the cited documents to use a novel sensor in the preparation of the cartridge of claim 35, and claim 35 is unobvious.

For the above reasons it is respectfully submitted that claims 22-27, 29-31 and 35-39 define patentable subject matter as do allowable claims 28 and 32-34.


Reconsideration and favorable action are solicited. If the examiner requires further information, please contact the undersigned.

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Respectfully submitted,

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